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ISOLATION AND MOLECULAR IDENTIFICATION OF PESTICIDES TOLERANT BACTERIA IN AGRICULTURE SOIL OF RAMPUR (U.P)

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ABSTRACT

Huge amounts of pesticides are applying every year in agriculture sector which help to increase the yield of crop which facilitate to fulfill the demand of food of increasing population. Pesticides controls harmful effects caused by the mark organisms. However, pesticides may unconsciously be transferred to the environment where they can impact non-target organisms. Micro organism in the soils developed capability to bioremediation of hazardous pesticides. In this work our aim is to identify pesticides tolerant bacteria in the agriculture soil. We collected the soil samples of vegetable filed. Isolation of pesticides tolerant bacteria were carried out using enrichment culture techniques containing pesticides as a sole source of carbon was isolated and selected for their ability to resistance against pesticides and antibiotics. We Identified 51 pesticides tolerant microorganisms in which 30 were showing Pseudomonas spps and remain were E.coli. Among 51, nine isolates were showing high pesticides tolerant.

KEYWORDS: Pesticides, Bacteria, Antibiotics & Bioremediation

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INTRODUCTION

Millions of tons of pesticides are applied every year in agriculture sector to the harmful effects caused by the target organisms including insects, fungi, bacteria, viruses etc **(Liu & Xiong 2001).** Pesticides can reach water through runoff from treated plant and soil. The pesticides leach down to soil and contaminate the ground water **(Kookana et al., 1998)** or if immobile, they would keep on the top soil where it could accumulate to toxic level in the soil and become harmful to microorganisms **(Amakiri 1982).** For example, long term low dose exposure to pesticide causes metabolism disorder, hormonal disruption, reproductive abnormalities and carcinoma **(Gupta, 2004).** Along with this their involvement into the food chain affects ecosystems and human beings **(Liu & Xiong 2001)**.

Sometimes pesticides decomposed into other chemical components, which become more toxic than the original pesticides. Therefore, these toxic compounds have been implicated in various disorders and diseases including cancer, adverse reproductive outcomes, peripheral neuropathies, neurobehavioral disorders, impaired immune functions and allergic sensitization reactions, particularly of the skin, cumulative inhibition of cholinesterase activity because of long-term low doses of exposure **(Okon, 1985).**

Bioremediation is the process in which microorganisms minimize the environmental hazardous wastes. Bioremediation is a promising alternative to physio-chemical methods of remediation, because it is less expensive and can selectively achieve complete destruction of organic pollutants **(Alexander, 1999).** A micro organism is an efficient tool to detoxify/decontaminate numerous toxic xenobiotics, especially pesticides (**Mervat 2009).** Polluted water and soil with pesticides can be biodegraded and is the primary mechanism of pesticide breakdown and detoxification **(Surekha et al., 2008).**

The tremendous increase of the population in the world causes higher demand of food; consequently it has led to sustainability of food production through improvement of agriculture with advanced agricultural technology **(Chowdhary et al., 2008).**

MATERIALS & METHODS

Collection of Soil Samples

Samples were collected applying the following methods: Approximately 1gm of Soil sample was collected between 0-15 cm surface of earth from the vegetable field of village of Rampur in dried sterilize begs and leveled properly. The soil was processed to make it free from any kind of dirt or other organic matter. Five soil samples were collected from different sites of vegetable field of Rampur village, (samodiya). Lumps present in soil samples were broken and all stones and debris were removed. Soil samples were than air dry, crushed and sieved through mesh.

Isolation and Purification of Isolates

Isolation of pesticide tolerant bacteria from soil sample were be done according to methods describe by **(Nawab et al., 2003**) on minimal media. Individual colonies of bacteria varied in shape size and color were picked up and purified on nutrient agar plates. After morphological characteristic of bacterial colony culture were identified on the microscopic (shape, size, color, margin, elevation, opacity, consistency, appearance of colony) and microscopic gram staining examination. Isolation of pesticide tolerant bacteria was done by serial dilution agar plate method. One gram of each soil sample were dissolved in 9ml of sterile water and mixed thoroughly. The supernatant of these suspensions was used further. 1 ml of suspension was transferred to 9 ml of sterile distilled water to get serially diluted10^{-1} to 10^{-5} 0.1 ml of suspension was spreader on the surface of minimal media ($10^{-3}$and 10^{-4}) with the help of spreader and incubates at 37 $^{\circ}$C for 24-48 hrs.

Biochemical Characteristic of Pesticides Tolerant Bacterial Isolates

Biochemical characterization of pesticides tolerant isolates was carried out by Hi Assorted ™ biochemical test kit (KB002-20 KT). Biochemical test kit was used for screening of pathogenic/non pathogenic organisms from environmental samples and other relevant samples. The kit provides the complete list of organism that can be identified with this system is given in the identification index provided with the kit. Each Hi Assorted ™ biochemical test kit is a standardized calorimetric identification system utilizing seven conventional biochemical test and five carbohydrate utilizing test. The tests are based on the principle of pH change and substrate utilization. On incubation organism undergo metabolic changes which are indicated by a color change in the media that can be either interpreted visually or after addition of the reagent.

16S RDNA Based Molecular Identification of Pesticides Tolerant Bacterial Isolates

Molecular characterization of pesticides tolerant isolates were carried out by according to protocol adopted from **Loffler et al., 2000.** Genomic DNA of each pesticides tolerant bacteria individually were isolated by methods describe in the **Sambrook et al., 2008**. Universal primers of 16s rDNA gene were designed with the help of primer 3 software with slight modification. Molecular identification of isolates were carried out by the amplification, sequencing and blast analysis of 16s rDNA gene with the help of Phylogenetic tree.

Universal Reverse primer	**5-GGTTACCTTGTTACGACTT-3**
Universal Forward primer	**5- AGAGTTTGATCCTGGCTCAG-3**

16S rDNA gene sequence were amplified with the help of PCR using universal primers at the following thermo cycler at 30 cycle at following programmed at 95°C at 20 min followed by 52 $^{\circ}$C at 5 min and then chain termination at 72 $^{\circ}$C at 10 min to stop the reaction.

Minimum Inhibitory Concentration (MIC) of Pesticide

Pesticides (Chlorpyrifos 50%: Meerut Agro Chemical industries limited, Meerut, Cypermethrin 25%: Shivalik Agro chemicals (J&k) and Triazophos 40 %: Kirshi Rasayan exparts New Delhi) tolerant bacteria was studied by the method adopted from **Gupta et al., 1994.** Concentration of the pesticide was calculated on the basis of methods describe by **Shafiani & Malik 2003.** Pesticides were supplemented it in NA media in varying concentration from 1% - 5% and then poured in Petri plates for polymerization. Spot inoculation was performed with the help loop and incubated at 23-37^{0}C for 24-48 hrs. The minimum concentration of the pesticide which inhibited the growth of the organism was considered as Minimum inhibitory concentration (MIC).

Antibiotic Susceptibility of Pesticides Tolerant Strain

The antimicrobial sensitivity of the test strains of six antibiotics was done using the Kirby-Bauer disk diffusion method **(Bauer et al., 1966).** The commercial available antibiotic discs were used for the study were tetracycline (5mg/ml), amoxicillin (5mg/ml), ofloxacin (4mg/ml), cefadroxil (5mg/ml), erythromycin (5mg/ml), and ampicillin (5mg/ml). Antibiotics were sterilized with Millipore filter membrane before preparation of different concentration of antibiotics and bacterial isolates were tested for sensitivity to antimicrobial agents by means of disc diffusion methods. A lawn of test pathogen was prepared by evenly spreading 100µl inoculums with the help of a sterilized spreader onto the entire surface of the agar plate. The plates were allowed to dry before applying antibiotic disc. Antibiotic discs were smoothly placed on the agar plates, and left at room temperature for 1 h to allow proper diffusion of the antibiotics into the agar medium. The plates were placed in incubator at 37°C for 24 hours for proper growth.

RESULTS & DISCUSSIONS

Five different sites (vegetable field) were selected for the isolation and identification of bacteria from the vegetable field of Rampur village and were identified on the basis of morphological, cultural, biochemical and molecular. More than 100 bacterial colonies were picked randomly from plates supplemented with different pesticides and finally 51 isolates were selected for further study. Minimum inhibitory concentration (MIC) of each isolates was determined with commercial pesticides Cypermetherin, Chlorpyrifos and Triazophos. Total 51 bacterial isolates (30, *pseudomonas* and 21 *E. coli*) were isolated from vegetable field of Rampur (Figure 1). All isolates showing gram negative rods and catalase positive. Among 51 isolates 30 isolates were showing as a *pseudomonas* while remain 21 isolates was *E. coli* in

biochemical characterization. In each soil sample mostly found gram negative bacteria. *Pseudomonas* gave the positive citrate utilization and glucose while Lysine and H_2S production were showing negative. *E.coli* gave the positive lysine, nitrate reduction and glucose while phenylalanine deaminase, H_2S production and Urease were showing negative test (Table 1).

Molecular identification were carried out on the basis of genomic DNA acting as template strand and universal primer attached on it and amplify 16S rDNA gene using suitable PCR cycling parameters. Method of 16S rDNA amplification is earlier described in materials and methods. The morphological and physiological characteristic of the isolates are given in the table 4 and 5. Among 51 only nine (Isolate 1, 9, 13, 18, 21, 31, 36, 40 and 45) isolates were molecular identified on the fact they were showing the growth at high concentration of pesticides.

The BLAST analysis of 16S rDNA sequences of nine bacterial isolates revealed that *E.coli* and *Pseudomonas*. Among nine isolate four (31, 36, 40 and 45) were showing *E.coli* and five (1, 9, 13, 18, 21) were showing *pseudomonas,* in which isolates 1 and isolates 9 was *Pseudomonas aeruginosa,* 13 and 18 were showing *Pseudomonas fluorescens* while isolate 21 were showing *Pseudomonas putida).*

Bacterial isolates were tested for tolerance to the pesticides: Chlorpyrifos, Cypermethrin and Triazophos. *Pseudomonas* and *E. coli* isolates were tolerated the maximum concentration of different pesticide such as 1% of Triazophos, 3% of Chlorpyrifos of and 4 % of Cypermethrin (Figure 2,3 & 4). Number of *Pseudomonas* isolates being resistance 100%, 90%, 70%, and 56.66% at various concentration of Cypermetherin 1%, 2%, 3% and 4% respectively (Table 3). At Chlorpyrifos experiment *pseudomonas* being resistance 100%, 46.66%, and 10% at various concentrations of Chlorpyrifos: 1%, 2% and 3% respectively while on Triazophos all *pseudomonas* were showing the growth only on 1% of concentration (Table 3).

E.coli isolates being resistance 100%, 90.47%, 28.57% and 19.04% at various concentration of Cypermetherin 1%, 2%, 3% and 4% respectively. *E.coli* being resistance 100%, and 38.09%, at various concentrations of Chlorpyrifos of 1%, and 2%, respectively (Table 4). While in Triazophos all *E.coli* were showing the growth only on 1% of concentration. **(Shafiani and Malik 2003)** tested Pseudomonas isolates tolerated concentration up to 1600 µg of all the three pesticides. **(Nawab et al., 2003)** reported that all pesticide tolerant bacterial isolate from contaminating soil were resistant to one or more antibiotics. The lowest concentration of pesticides/antibiotics inhibiting the growth of bacteria was considered as Minimum Inhibitory Concentration (MIC).

Micro organism adapt to change environmental condition by horizontal gene transfer which provides them with new traits (pesticides/antibiotics/metal resistance) so they can survive and colonize their new environments **(De Gelder et al., 2008).** In this context we studied nine pesticides tolerant bacteria were tested for antibiotic (Tetracyclin, ofloxacin, ampicillin, erythromycin, amoxicillin and cefadroxil) susceptibility means of disc diffusion methods **(Bauer et al., 1966).** We tested nine isolates for MIC of six different antibiotics (Tetracyclin, ofloxacin, ampicillin, erythromycin, amoxicillin and cefadroxil) of its various concentrations 0.035 to 5.0mg/ml. Isolates 1 (*Pseudomonas aeruginosa,)* were showing the MIC 0.15mg/ml and 0.6mg/ml at two different antibiotics Ofloxacin and Tetracyclin respectively while resistance against, amoxicillin, erythromycin and cefadroxil. Isolates 9 (*Pseudomonas aeruginosa)* is also showing the MIC 0.15mg /ml for Tetracyclin. Isolates 21 (*Pseudomonas putida*) 31 (*E.coli*) 36 (*E.coli*) and 45(*E.coli*) were showing the similar MIC value as we found in isolates 1 (*Pseudomonas aeruginosa*) is 0.15mg/ml for ofloxacin while isolates 13(*Pseudomonas flurorescens*) were showing low MIC value 0.07mg/ml for ofloxacin (Figure 5).

Isolates 1 (*Pseudomonas aeruginosa)*, 21 (*Pseudomonas putida*), 36(*E. coli*) and 45(*E. coli*) were showing the same MIC value 0.6mg/ml for Tertracyclin while isolates 13 (*Pseudomonas flurorescens*) 31(*E. coli*) and 40(*E. coli*) were showing the MIC 0.3gm/ml on the same antibiotics. MIC value 0.15mg/ml were found for isolates 9 (*Pseudomonas aeruginosa)* and 18 (*Pseudomonas flurorescens)* on the above antibiotics. Maximum MIC value showing 5mg/ml of isolates 18(*Pseudomonas flurorescens*) for Amoxicillin (Figure 5).

We found that three different isolates i.e. Isolates9 (*Pseudomonas aeruginosa)* Isolates 18 (*Pseudomonas flurorescens)* and Isolates40 (*E.coli*) is highly sensitive against ofloxacin while isolate 36 (*E.coli*) and isolate 1(*Pseudomonas aeruginosa)* is highly sensitive for Erythromycin and ampicillin respectively. Among nine isolates all were showing the resistance for erythromycin except isolates 36 (*E.coli*) while for ampicillin all were resistance except isolates1 (*Pseudomonas aeruginosa)*. For amoxicillin all were resistance except isolate18 (*Pseudomonas flurorescens*) while cefadroxil all isolates were showing resistance.

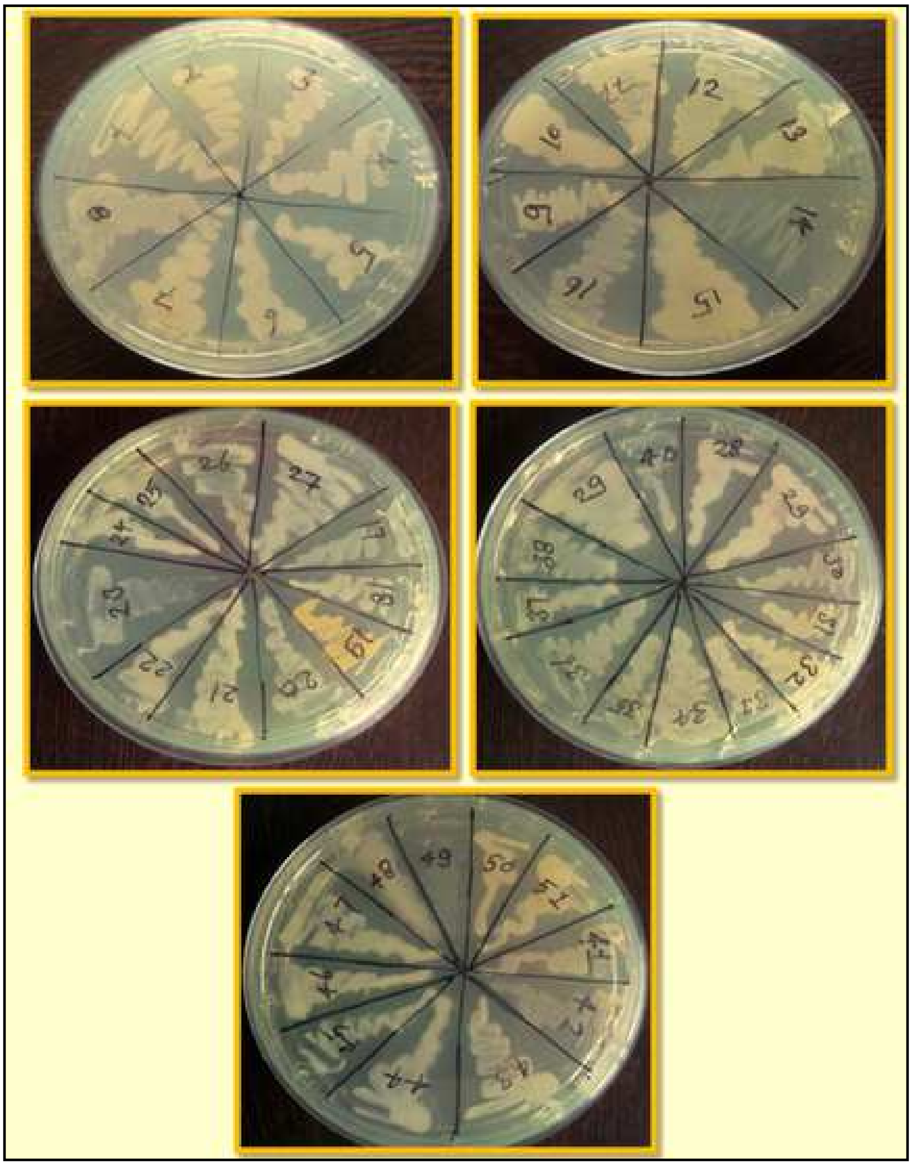


Figure 1: Purification of Bacterial Isolates

Table 1: Biochemical Characterization of Isolates *Pseudomonas* (30) Obtained from Vegetable Field of Rampur

Biochemical Characterization of Isolates																															
No.	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	Citrate Utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Lysine Utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Ornithine Utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Urease	V	v	+	v	+	+	v	v	+	+	v	+	+	v	+	+	V	v	+	v	V	+	+	+	v	+	v	v	+	V
5	Phenylalanine Deamination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Nitrate Reduction	+	+	+	+	+	+	+	+	+	+	v	+	+	v	+	v	V	+	V	+	-	-	-	-	-	-	-	-	-	-
7	H_2S Production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	Adonitol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd
10	Lactose	-	-	-	-	-	-	-	-	-	-	v	V	+	v	+	+	V	+	V	v	V	+	+	+	v	+	+	v	v	+
11	Arabinose	-	-	-	-	-	-	-	-	-	-	v	+	+	v	+	v	+	+	v	+	V	+	V	V	+	+	V	v	+	+
12	Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nd	nd	nd	Nd	nd	nd	nd	nd	nd	nd

Table 2: Biochemical Characterization of Isolates *E. coli* (21) Obtained from Vegetable Field of Rampur

Biochemical Characterization of Isolates																						
No.	Sample	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
1	Citrate Utilization	V	+	+	v	v	+	+	+	+	+	V	V	+	V	+	+	+	v	V	+	+
2	Lysine Utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Ornithine Utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Phenylalanine Deamination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	H_2S Production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

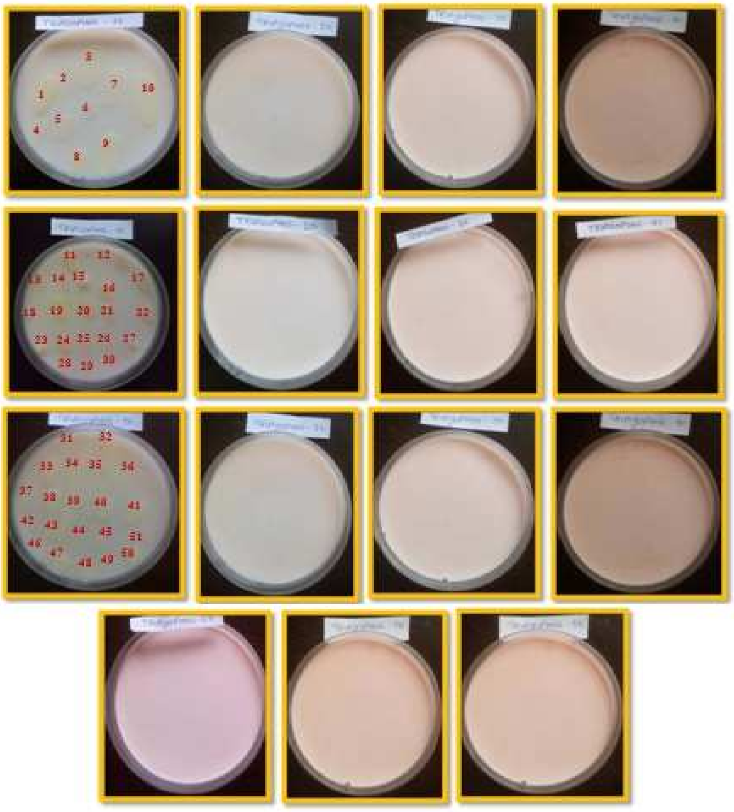


Figure 2: Minimum Inhibitory Concentration (MIC) of Triazophos under the Influence

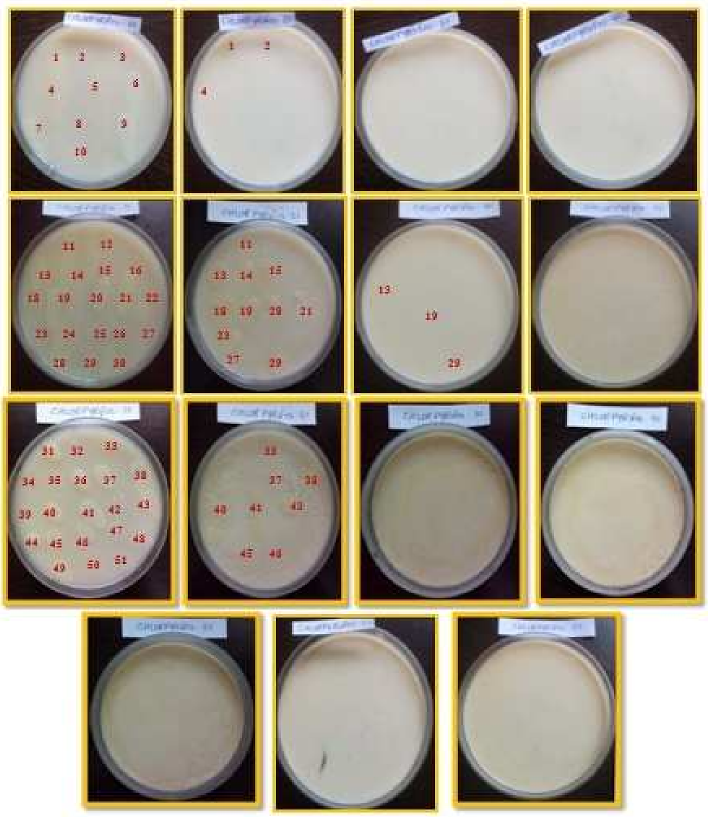


Figure 3: Minimum Inhibitory Concentration (MIC) of Chlorpyrifos under the Influence of Following Bacterial Isolates (Isolates 1 - 30: *Pseudomonas*) and (Isolates 31-51: *E. coli*)

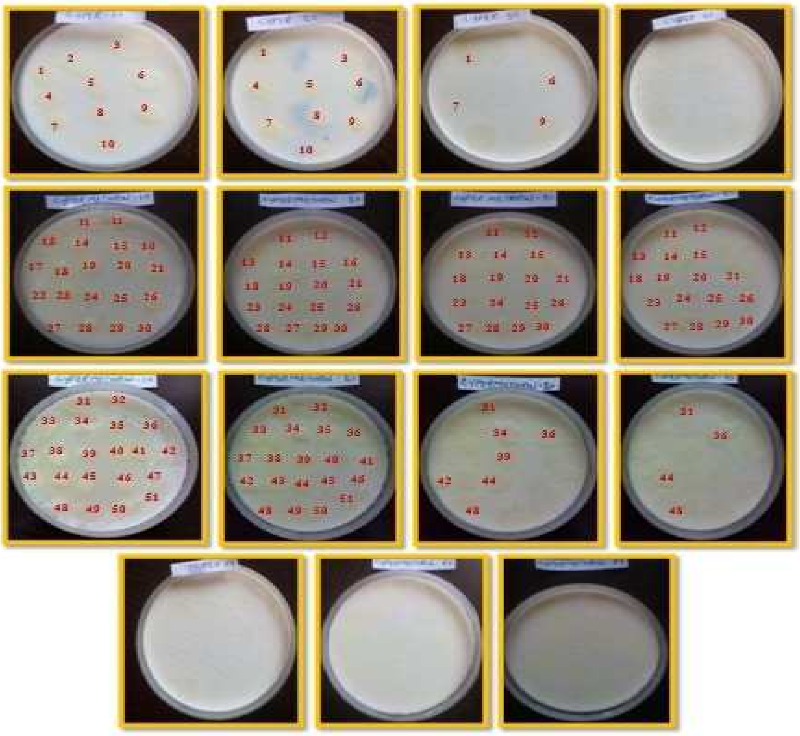


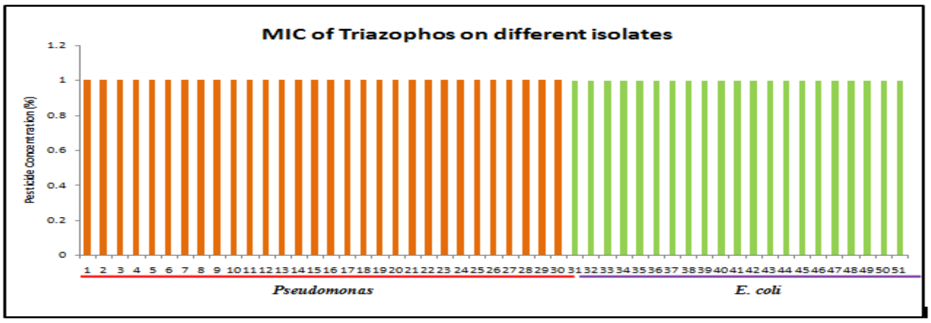
Figure 4: Minimum Inhibitory Concentration (MIC) of Cypermethrin under the Influence of Following Bacterial Isolates (Isolates 1- 30: *Pseudomonas)* and *(*Isolates 31-51: *E. coli)*

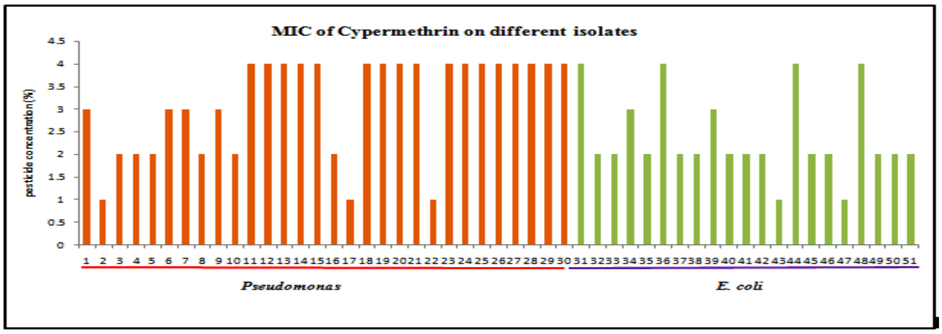
Table 3: Total Percent of *Pseudomonas* (30) Isolates Being Inhibited/ Resistance against Different Pesticide

Total Percent of Pesticides Tolerant Pseudomonas (30) Being Inhibited/ Resistance against Different Pesticide			
Name of Pesticide	**Concentration (%)**	**No. of Isolates Being Inhibited**	**No. of Isolates Being Resistant**
Cypermethrin	1	0(0)	30(100)
	2	3(10)	27(90)
	3	9(30)	21(70)
	4	13(43.33)	17(56.66)
	5	30(100)	0(0)
Chlorpyrifos	1	0(100)	30(100)
	2	16(53.33)	14(46.66)
	3	27(90)	3(10)
	4	30 (100)	0(0)
	5	30(100)	0(0)
Triazophos	1	0(0)	30(100)
	2	30(100)	0(0)
	3	30(100)	0(0)
	4	30(100)	0(0)
	5	30(100)	0(0)

Table 4: Total Percent of *E. coli* (21 Isolates) Being Inhibited/ Resistance against Different Pesticide

Total Percent of Pesticides Tolerant E.Coli (21) Being Inhibited/ Resistance Against Different Pesticide			
Name of Pesticide	**Concentration (%)**	**No. of Isolates Being Inhibited**	**No. of Isolates Being Resistant**
Cypermethrin	1	0(0)	21(100)
	2	2(9.52)	19(90.47)
	3	15(71.42)	6(28.57)
	4	17(80.95)	4(19.04)
	5	21(100)	0(0)
Chlorpyrifos	1	0(100)	21(100)
	2	13(61.90)	8(38.09)
	3	21(100)	0(0)
	4	21 (100)	0(0)
	5	21(100)	0(0)
Triazophos	1	0(0)	21(100)
	2	21(100)	0(0)
	3	21(100)	0(0)
	4	21(100)	0(0)
	5	21(100)	0(0)





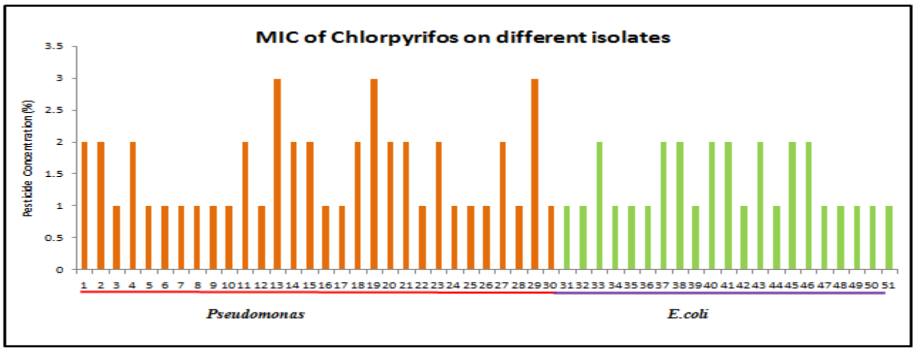


Figure 5: Graph Representing the Minimum Inhibitory Concentration (MIC) of Triazophos, Chlorpyrifos and Cypermetherin on Different Isolates

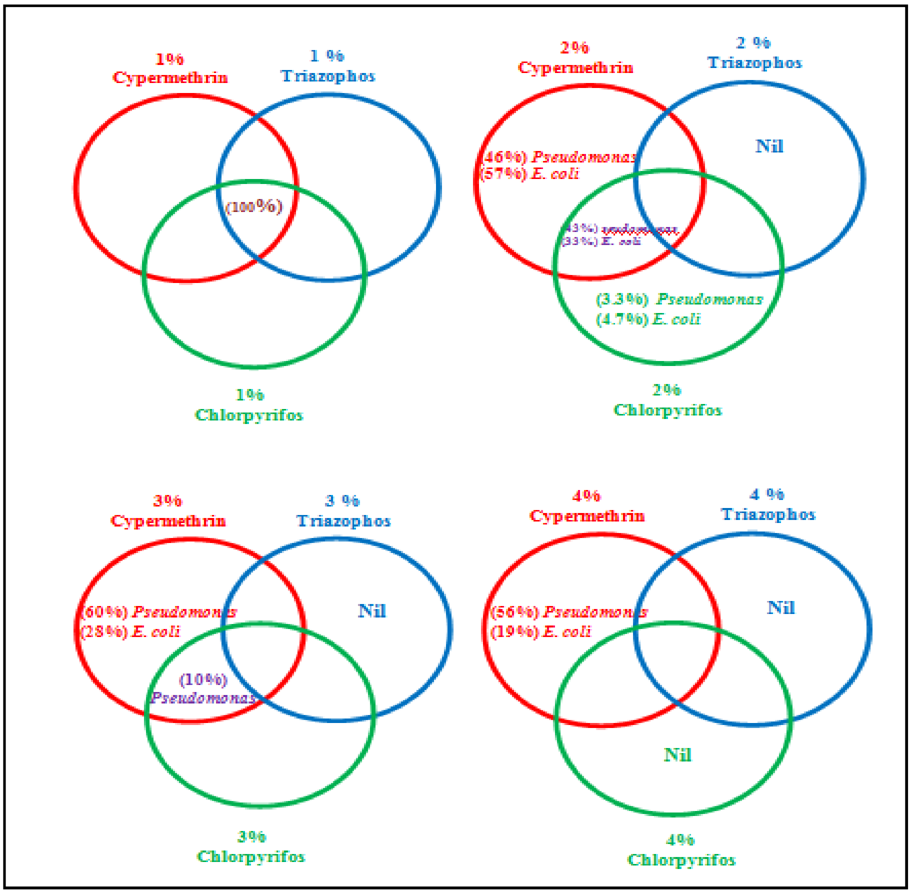


Figure 6: Vein Diagram Representing the Pesticides (Triazophos, Chlorpyrifos and Cypermetherin) Tolerating Common Isolates of *Pseudomonas* and *E.coli* in at Different Concentration Varies at 1% to 4%

Table 5: Zone of Inhibition of Antibiotic at Different Concentration under the Influence of Isolates 1, 9, 13, 18, 21, 31, 36, 40 and 45

		Zone of Inhibition (cm)								
Name of Antibiotic	**Concentration (mg/ml)**	**Isolate No. 1**	**Isolate No. 9**	**Isolate No. 13**	**Isolate No. 18**	**Isolate No. 21**	**Isolate No. 31**	**Isolate No. 36**	**Isolate No. 40**	**Isolate no. 45**
Ofloxacin	5mg/ml	2.4 cm	2.5 cm	2.6 cm	2.1 cm	2.1 cm	2.4 cm	2.9 cm	2.5 cm	1.9 cm
	2.5 mg/ml	2 cm	2.3 cm	2.4 cm	1.9 cm	1.8 cm	2 cm	2.2 cm	2.0 cm	1.3 cm
	1.25mg/ml	1.7 cm	2 cm	1.9 cm	1.6 cm	1.5 cm	1.5 cm	1.9 cm	1.7 cm	1.1 cm
	0.6mg/ml	1.5 cm	1.8 cm	1.7 cm	1.3 cm	1.3 cm	1.1 cm	1.4 cm	1.5 cm	0.9 cm
	0.3mg/ml	0.8 cm	1.5 cm	1.4 cm	1.1 cm	1 cm	0.9 cm	1 cm	1.2 cm	0.7 cm
	0.15mg/ml	0.6 cm	1.3 cm	1 cm	1.0 cm	0.9 cm	0.7 cm	0.8 cm	1.0 cm	0.5 cm
	0.07mg/ml	-	1.1 cm	0.7 cm	0.9 cm	-	-	-	0.8 cm	-
	0.035mg/ml	-	0.8 cm	-	0.7 cm	-	-	-	0.5 cm	-
Erythramycin	5mg/ml	--	--	--	--	--	--	1.8 cm	--	--
	2.5 mg/ml	--	--	--	--	--	--	1.5 cm	--	--
	1.25mg/ml	--	--	--	--	--	--	1.2 cm	--	--
	0.6mg/ml	--	--	--	--	--	--	1.0 cm	--	--
	0.3mg/ml	--	--	--	--	--	--	0.9 cm	--	--
	0.15mg/ml	--	--	--	--	--	--	0.8 cm	--	--
	0.07mg/ml	--	--	--	--	--	--	0.6 cm	--	--
	0.035mg/ml	--	--	--	--	--	--	0.5 cm	--	--
Ampicillin	5mg/ml	2.8 cm	--	--	--	--	--	--	--	--
	2.5 mg/ml	2.4 cm	--	--	--	--	--	--	--	--
	1.25mg/ml	2.2 cm	--	--	--	--	--	--	--	--
	0.6mg/ml	2.0 cm	--	--	--	--	--	--	--	--
	0.3mg/ml	1.8 cm	--	--	--	--	--	--	--	--
	0.15mg/ml	1.5 cm	--	--	--	--	--	--	--	--
	0.07mg/ml	1.3 cm	--	--	--	--	--	--	--	--
	0.035mg/ml	1.0 cm	--	--	--	--	--	--	--	--

Table 6: Zone of Inhibition of Antibiotic at Different Concentration under the Influence of isolates 1, 9, 13, 18, 21, 31, 36, 40 and 45

		Zone of Inhibition (cm)								
Name of Antibiotic	**Concentration (mg/ml)**	**Isolate No. 1**	**Isolate No. 9**	**Isolate No. 13**	**Isolate No. 18**	**Isolate No. 21**	**Isolate No. 31**	**Isolate No. 36**	**Isolate No. 40**	**Isolate No. 45**
Tetracyclin	5mg/ml	1.7 cm	1.9 cm	1.7 cm	1.4 cm	1.3 cm	1.4 cm	1.4 cm	1.6 cm	1.5 cm
	2.5 mg/ml	1.1 cm	1.6 cm	1.5 cm	1.2 cm	1.1 cm	1.2 cm	1.2 cm	1.2 cm	1.2 cm
	1.25mg/ml	1 cm	1.2 cm	1.1 cm	1.1 cm	0.9 cm	1 cm	0.8 cm	1 cm	0.8 cm
	0.6mg/ml	0.8 cm	1 cm	0.9 cm	1 cm	0.7 cm	0.7 cm	0.5 cm	0.8 cm	0.5 cm
	0.3mg/ml	-	0.8 cm	0.7 cm	0.8 cm	-	0.5 cm	-	0.5 cm	-
	0.15mg/ml	-	0.5 cm	-	0.5 cm	-	-	-	-	-
	0.07mg/ml	-	-	-	-	-	-	-	-	-
	0.035mg/ml	-	-	-	-	-	-	-	-	-
Amoxicillin	5mg/ml	--	--	--	0.7 cm	--	--	--	--	--
	2.5 mg/ml	--	--	--	-	--	--	--	--	--
	1.25mg/ml	--	--	--	-	--	--	--	--	--
	0.6mg/ml	--	--	--	-	--	--	--	--	--
	0.3mg/ml	--	--	--	-	--	--	--	--	--
	0.15mg/ml	--	--	--	-	--	--	--	--	--
	0.07mg/ml	--	--	--	-	--	--	--	--	--
	0.035mg/ml	--	--	--	-	--	--	--	--	--
Cefadroxil	5mg/ml	--	--	--	--	--	--	--	--	--
	2.5 mg/ml	--	--	--	--	--	--	--	--	--
	1.25mg/ml	--	--	--	--	--	--	--	--	--
	0.6mg/ml	--	--	--	--	--	--	--	--	--
	0.3mg/ml	--	--	--	--	--	--	--	--	--
	0.15mg/ml	--	--	--	--	--	--	--	--	--
	0.07mg/ml	--	--	--	--	--	--	--	--	--
	0.035mg/ml	--	--	--	--	--	--	--	--	--

CONCLUSIONS

The result of our study revealed that not only *pseudomonas* but also *E.coli* has adaptation for bioremediation and ability to survive at various concentrations of pesticides. We know that continuously pesticides are using for the enhancement in crop yield but it is not necessary that they can have the positive impact on the crops and animals. A large number of diseases (Cancer, Diabetes) increasing among humans. Biodegradation is becoming a method of choice for the remediation of polluted site. In our study we characterized pesticide tolerant bacteria from agriculture soil that was highly contaminated with the pesticides (Triazophos, Cypermethrin, and Chlorpyrifos) due to frequently use of pesticides for vegetable production. High/low MICs of bacterial isolates shows that resistant or tolerant to the pesticides, they are simultaneously resistant to antibiotics also. In this context the results of the present study suggest that the isolates are able to grow in presence of pesticides due to its bioremediation property.

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